A Disposable DNA Sample Preparation Microfluidic Chip for Nucleic Acid Probe Assay

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ABSTRACT

A new DNA sample preparation microfluidic chip for Nucleic Acid (NA) probe assay has been proposed. The proposed microfluidic chip is composed of three parts: microfilter, micromixer and DNA purifiction chip. We have fabricated a microsieve type filter with an array of 2.2μm diameter holes. We have also demonstrated the mixing can be successfully achieved for low Reynold numbers below 50 by using the fabricated micromixer. The fabricated DNA purification chip has shown a binding capacity of 15ng/cm^2 and a minimum extractable input concentration of 100copies/200μL. The proposed microfluidic chip can be applied for low-cost, disposable sample preparation of NA probe assays.

INTRODUCTION

Nucleic acid (NA) probe assays have been enormous scope of applications in biotechnology and medicine, ranging from agriculture and farming to the detection of pathogens in foods to genetic diagnostics on human subjects [1,2]. Recently, there has been much interest in the implementation of microfluidic devices for NA probe assays. Theses devices are excellent candidates for miniaturization because the performance and costs of NA probe assays can be improved in the microsacle and the same microfabricated part can be used for many different assays by changing the natures of its reagents, not the devices construction [3].

The purpose of NA probe assays is the detection and reporting of very small amounts of predetermined NA sequences in biological fluid samples. The NA probe assays typically include PCR to amplify the number of copies of DNA to a detectable level. The PCR technique requires a relatively pure DNA sample in aqueous solution, free of inhibitors during the PCR process. Therefore, the extraction and purification of nucleic acids from biological samples are the critical steps that should be carefully handled in the NA assays [4]. Also, this series of complex chemical processes are the most difficult and time-consuming part.

In this paper we have proposed a new microfluidic chip to address this sample preparation process for NA probe assays. The proposed microfluidic system is composed of three parts: microfilter, micromixer and DNA purification chip (Fig.1). In the microfilter, red blood cells are separated from whole blood sample. In the mixer, the plasma blood sample is mixed with lysis reagents to release the DNA into the solution and the lysed solution is mixed with chaotropic salt. In the DNA purification chip, the DNA's in the solution will bind to the exposed SiO_2 surface at a high concentration of chaotropic salt [5,6]. Photosensitive glass has been chosen as a binding substrate because its process is simple and inexpensive compare to other alternative technologies such as silicon deep RIE technique [7].

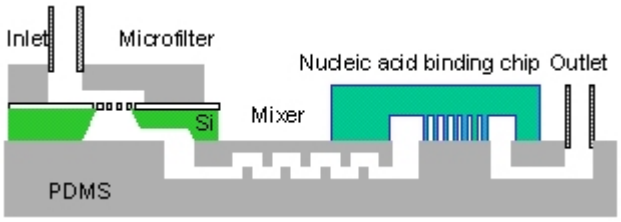


Figure 1. *Proposed microfluidic system for sample preparation of Nucleic Acid probe assay.*

DESIGN AND FABRICATION

Microfilter using electroplated Ni

We have designed the microfilter to separate red blood cells from whole blood sample because red blood cells are known to be a major inhibitor in PCR process. Red blood cells cannot pass through a gap smaller than 3μm [4]. The proposed microfilter has a metallic microsieve, as shown in Fig. 2.

The microstructures have been fabricated using thick photoresist (PR) mold and electroplating technology [8]. For the thick photoresist, we used the AZ9260 manufactured by Hoechst. The micro filter is formed on a p-type (100) silicon wafer. Silicon nitride is deposited and patterned as a mask layer for the KOH etching of the bulk silicon in the later process step. Next, a seed layer (Cr/Au) is evaporated followed by the definition of the first

photoresist mold of 20μm in thickness and nickel is electroplated. After photoresist mold is removed, bulk silicon is etched in KOH and the seed layer is removed. Finally, the substrate is bonded to a polydimetylsiloxane (PDMS) cover plate to build closed channels. Fig. 3 shows the patterned thick PR mold for the metallic microsieve and the fabricated Ni microstructure, which has an array of 2.2μm diameter holes.

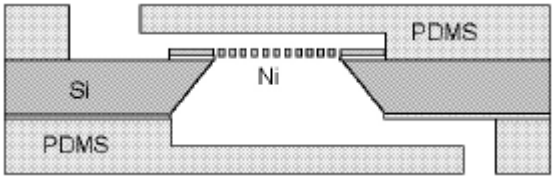


Figure 2. *Schematic diagram of the proposed microfilter.*

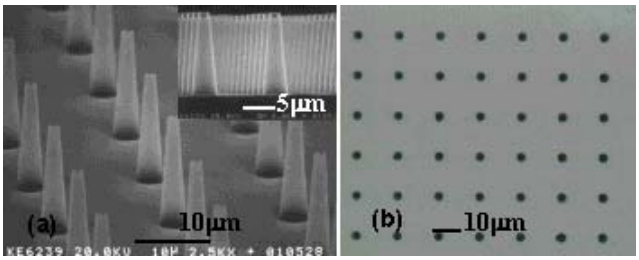


Figure 3. *(a) SEM picture of the fabricated thick PR (AZ9260) mold, (b) Microphotograph of the fabricated microfilter with a hole diameter of 2.2μm.*

Passive Micro Mixer

It has been shown that a "twisted pipe" has the potential to enhance mixing even at low Reynolds numbers (<100). This mixing enhancement is possible because of the phenomenon known as chaotic advection [9], in which simple regular velocity fields produce chaotic particle trajectories. The occurrence of chaotic advection typically indicates rapid distortion and elongation of material interfaces. This process significantly increases the area across which diffusion occurs, which leads to rapid mixing.

In order to mix the liquid well at low Reynolds numbers, the geometry of a channel must be "complicated enough" that chaotic advection can be triggered. However, in order for fluidic channels to be easily fabricated and integrated into the other microfluidic systems, the geometry should remain "relatively simple".

The proposed mixer consists of two-layer channels. Mixing is a two-step process. The first step is segmentation where a heterogeneous mixture of two fluids is formed by convection; and the second step is the inter-diffusion of molecules between domains. As shown in Fig. 4, the proposed mixer has a separated serpentine flow path in order to increase the chaotic advection as well as has the repeated segments to increase the interfacial area.

The micro mixer has been fabricated using PDMS. The master for channel mold has been patterned on silicon wafer by using the SU-8. Next, the PDMS prepolymer mixture (curing agent: PDMS prepolymer=1:10) is poured onto the master. After curing for 90min at 85°C, the PDMS replicas are peeled off from the master. Finally, two PDMS structures are bonded. The fabricated mixer is shown Fig. 5. The depth of the channel is about 100μm and the width is 400μm.

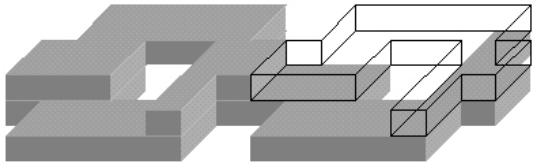
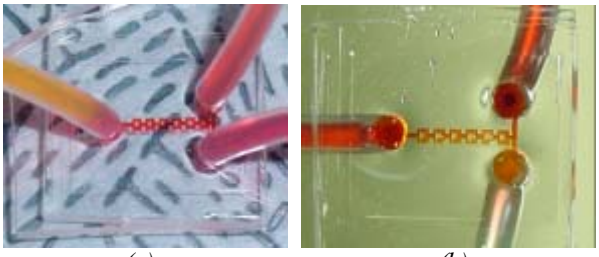


Figure 4. *Schematic diagram of the proposed micromixer.*



(a) *(b)*

Figure 5. *Photographs of fabricated micromixer (a) top view (b) bottom view*

DNA Purification Chip on Photosensitive Glass

In general, it is known that DNA binds to silica in the high concentration of chaotropic salt and elutes in low salt [5]. This characteristic can be used as a useful method as a purification procedure. Since the DNA is eluted with either water or a low salt buffer, the elution process can be immediately followed in a subsequent reaction on the same substrate posterior to the binding procedure.

The proposed DNA purification chip has been designed in order to maximize DNA binding surface area on a photosensitive glass substrate as shown Fig. 6. Photosensitive glass has been chosen as a binding substrate because its process is simple and inexpensive compared with other alternative technologies such as silicon deep RIE.

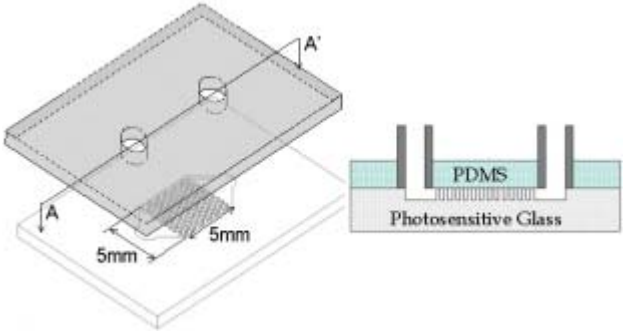


Figure 6. *Schematic diagram of DNA purification chip.*

DNA purification starts with the introduction of biological fluids into the microchannel of the fabricated chip. The biological fluids are mixed with an appropriate binding chemistry such as a chaotropic salt; therefore, DNA will naturally bind to the glass (SiO_2) surface. A flow of the biological fluid sample through the multiple microchannels will result in a gradual accumulation of NA on the large surface area. After DNA has been adequately extracted in the binding process, wash solutions flow through the microchannel of the chip to wash away the remaining sample fluid. Finally, the DNA can be eluted from the chip by flowing through appropriate chemical buffer solutions.

The first process step is to expose the substrates to an UV-light at about 300nm in wavelength. It is illuminated with an energy density of 2.5J/cm^2 at the intensity of 15mW/cm^2. Heat treatment is performed for 3 hours at 500~600°C after exposure. During this heat cycle, the exposed area will be crystallized and can be selectively dissolved in a solution of 10% hydrofluoric acid (HF) in an ultrasonic bath at room temperature. The etching rate of the exposed glass is about 10μm/min. After the etching process, the glass substrate is bonded to a PDMS cover plate to build closed channels.

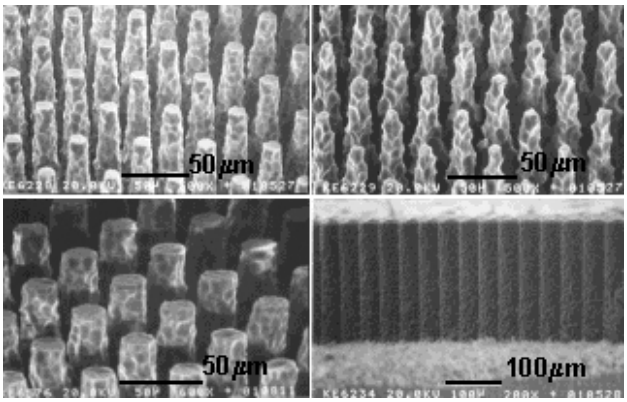
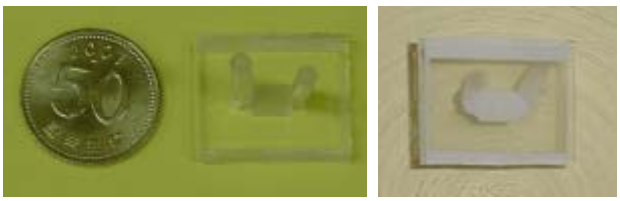


Figure 7. *SEM pictures of photosensitive glass microstructures.*



(a) (b)

Figure 8. *Photographs of the fabricated DNA purification chip: (a) top view, (b) bottom view.*

Fig. 7 shows the fabricated photosensitive glass substrate which will form multiple microchannels. The fabricated glass surface is consisted of a number of pillars with a height of 200μm. The pillar diameter is 25μm and its pitch is 50μm. The fabricated DNA purification chip is shown in Fig. 8. We have used the PMDS to fabricate the cover plate. After bonding to the PDMS top cover, the chip has a total internal surface area of 2cm^2 that will be effectively used as DNA binding sites.

RESULTS AND DISCUSSIONS

The proposed mixer has a two-way separated serpentine flow path in order to increase the chaotic advection as well as to increase the interfacial area. We have tested the mixer using phenolphthalein, a pH indicator that changes its color from transparent to red for higher pH values than 8 [10]. Fig. 9 shows the mixing of 0.1 mol/L phenolphthalein and 0.3mol/L NaOH in the micromixer connected in series. Fig. 10 shows the normalized average optical intensity in each stage of the fabricated mixer. After five or six mixing sections two streams were fully mixed.

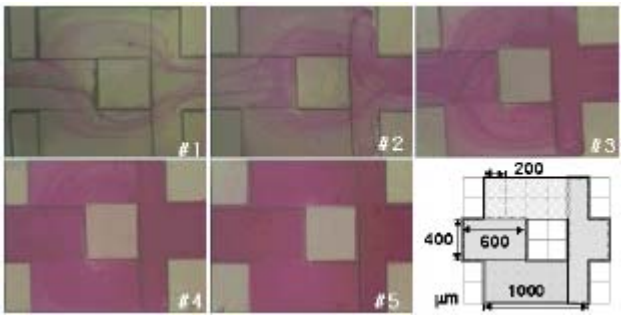


Figure 9. *Photographs at each consecutive segment stage of the fabricated mixer at a flow rate of 2mL/min.*

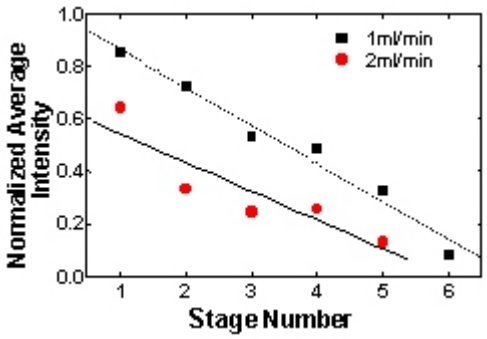


Figure 10. *Normalized average intensity in each stage of the mixer.*

For the DNA binding test studies of the fabricated chip, a fluidic test system consisting of a microsyringe pump and several tubes have been used. An experimental protocol is as follows as shown in Fig. 11. The conditions of used reagents are listed in Table 1.

The chaotropic DNA starting solution is first passed through the chip. During this procedure, the DNA present in the solution binds to the glass surface of the microchannels. Next, the ethanol-based wash solution is introduced. The wash solution is intended to wash away the salts and other PCR inhibitors that might be present in the sample. Finally, an elution reagent is introduced to the chip and is stayed for several minutes, releasing the

DNA back into the fluid stream. This solution is processed through gel electrophoresis with 1% agarose gels and ethidium bromide (EtBr) staining.

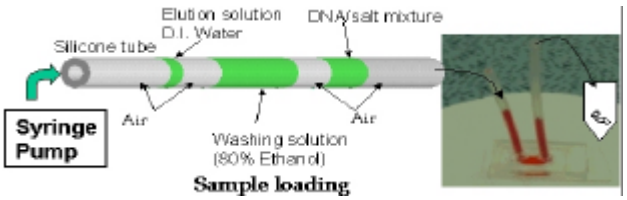


Figure 11. *Test bench of DNA purification chip.*

Table 1. *Condition of test reagents.*

Reagent	High concentration	Low concentration
Starting DNA	4780 bp plasmid dsDNA	
DNA/chaotropic salt mixture volume	600ng/200µl	100copies/200µl
Chaotropic salt concentration	4.8mol/L guanidine HCl	5.8mol/L guanidine HCl
Wash solution	400µl ethanol	
Elution solution	40µl D.I. Water	
PCR	No	30cycles

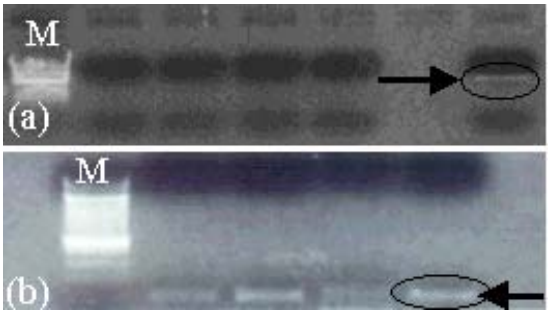


Figure 12. *Photograph of electrophoretic gel with EtBr staining showing the eluted DNA from the DNA purification chip; Lane M: Marker: (a) high concentration sample (600ng/200µl input) without PCR, (b) low concentration sample (100copies/200µl) with PCR amplification.*

We have tested two experiments. One is a high concentration input test (600ng/200µL) and the other is a low concentration test (100copies/200µL). For the high concentration sample, the presence of the target DNA can be detected without PCR. Fig. 12 (a) shows the photograph of electrophoretic gel showing the actual amount of the eluted DNA from the fabricated DNA purification chip. The amount of the released DNA has been detected about 30ng. This means that the binding capacity of the chip is about 15ng/cm^2. For the low concentration input test, PCR must be performed to amplify a target sequence in order to detect the presence of the target DNA. Fig. 12 (b) shows that the fabricated purification chip can detect the gel band of the eluted DNA from a low concentration input sample below 100copies/200µL. These characteristics demonstrate that the proposed DNA purification chip can be applied for the genomic diagnostic applications and the infectious disease diagnostic applications.

CONCLUSIONS

In this paper, we have proposed and fabricated a DNA sample preparation microfluidic chip for Nucleic Acid (NA) probe assay. We have monitored the mixing at various flow rates at each segment of the fabricated microchannel. In the micro scale, we have demonstrated that the fabricated mixer can achieve the mixing successfully for low Reynolds numbers below 50. We have successfully captured and eluted the DNA using the fabricated DNA purification chip that has a binding capacity of 15ng/cm^2 and a minimum extractable input concentration of 100copies/200µL. These characteristics have demonstrated that the proposed microfluidic chip can be applied for low-cost and disposable microsystems for the sample preparation of nucleic acid probe assays.

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